

~~Agent for treating septicemia, process for preparing and therapeutic process~~

~~Field of invention~~ [Therapeutic agent for the treatment of septicemia, its preparation and use]

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The invention relates to a therapeutic agent for the treatment of septicemia, its preparation and use ~~in the~~ [Fields of application of the invention are] pharmaceutical industry and ~~in~~ medicine.

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10 Background

Septicemia with its frequently lethal complications is one of the ~~most feared~~ clinical syndromes in medicine [feared most]. It is therapeutically not controllable, claiming ~~hundreds of thousands of~~ [a few 100,000] casualties a year [alone] in [the] Western countries ~~alone~~. New treatment options have to be ~~found because~~ [looked for as] antibiotics act too slowly, ~~and do~~ not ~~prevent~~ [preventing] the release of bacterial toxins ~~and~~ [,] partly even ~~intensify~~ [intensifying] it. Pouring out of messenger substances (cytokines) by the host organism released by bacterial toxins is the most important element of the pathogenetic cascade leading to the clinical ~~evolution~~ [picture] of septicemia. Various new therapeutic approaches, ~~as~~ [on the one hand,] blocking of bacterial lipopolysaccharide (LPS) as the most important toxin by antibodies or antagonizing the endogenous, so-called proinflammatory cytokines failed completely in comprehensive clinical studies (C. Natanson et al., Ann. Intern Med. 120, 771-783 (1994)).

25 ~~Summary of the~~ [The] invention [is based on the task]

~~It is an object of the present invention~~ to develop a therapeutic agent for the treatment of septicemia.

~~In accordance with the present invention, it was discovered that highly dosed LBP suppresses the synthesis of an important septicemia mediator molecule caused by the bacterial toxin LPS, namely TNF. The production of this TNF protein and other mediators is suppressed in the mouse by the addition of LBP. In addition, liver damage induced by the addition of LPS is prevented by LBP and the number of surviving animals significantly increases. That means that the addition of LBP protects against the effects of LPS during septicemia, which represents a new therapeutic process for treating septicemia. Thus, the above object was accomplished\ [This task was solved] by an agent containing the protein LBP binding lipopolysaccharide\, its mutants, variants or hybrid proteins, as basic active component. Human and murine such as rat, or rabbit LBP are also effective for the purpose.~~

~~Brief description of the drawing~~

~~The invention is disclosed with reference being had to the drawing, wherein:~~

~~Fig. 1 shows stimulation of a murine macrophage cell line in vitro with various concentrations of the bacterial toxin LPS for synthesizing the septicemia mediator TNF depending on LBP;~~

~~Fig. 2 shows how due to the LBP contained in serum, the addition of LBP in the presence of serum suppresses the production of TNF by the macrophage cell line;~~

~~Fig. 3 shows the suppression of TNF syntheses as the concentration of serum is increased with the added LBP remaining constant;~~

~~Fig. 4 shows that LBP levels of mouse produced by an exogenous addition of LBP correspond to the acute physiological phase levels produced by addition of LPS;~~

~~Fig. 5(a) shows with TNF that cytokine release induced by LPS can be suppressed in the mouse by the simultaneous addition of LBP;~~

Fig. 5(b) shows that with IL-6 cytokine release induced by LPS can be suppressed in the mouse by the simultaneous addition of LBP;

Fig. 6 shows that in addition, the liver damage caused by LPS and detected by increasing ALT enzyme levels, are suppressed by simultaneously adding LBP; and

Fig. 7 shows that the addition of LBP significantly reduces the lethality in a LPS septicemia model, carried out with 20 mice per group.

Detailed description\ [as basic component.

The characteristic features of the invention are contained claims 1-11.

It is possible to use also murine or rabbit LBP apart from human LBP.]

The structure of LBP is known. It was obtained by isolating a clone from an acute phase cDNA gene bank and subsequently sequencing and deriving the amino acid sequence. Recombinant LBP is prepared by cloning cDNA in an expression vector and co-infection of insect cells with the baculovirus.

The cloned protein (LBP) binds LPS with high affinity and is secreted into serum as acute phase protein during septicemia. As is shown in the figures, [examples] LBP inhibits the [LPS] effects of LPS and can [may] -if given to mice - suppress septicemia caused by LPS and very significantly reduce lethality [highly significantly]. This applies when using LBP simultaneously with the onset of septicemia and also before. Thus LBP appears [may seem] to be suited for preventing septicemia also in high-risk patients [also]. As LPS plays a central part also in septicemia caused by gram-positive bacteria and for the systemic inflammatory response syndrome, a clinical picture identical to septicemia caused by trauma, by [via] translocation of gram-negative intestinal flora. The [, the] inhibition of LPS effects by LBP can [may] also improve these dramatic clinical syndromes.

Apart from the highly active recombinant LBP optimized mutants the function of which was modified ~~and~~ which are also used as therapeutic agents for the treatment of septicemia~~\,~~ are equally suited for implementing the ~~present~~ invention.

A further basic possibility for implementing the ~~present~~ invention is to clone the LBP gene in an adenoviral vector with high activity in the liver behind the strong CMV promoter to achieve high levels of expression in addition to the intrinsic hepatic expression of LBP.

The invention ~~can be suitably applied~~ **[is applicable]** in the case of **[septicemia caused by gram-negative ~~and~~ **[bacteria septicemia caused by]** gram-positive bacteria\, and in the case of** systemic inflammatory response syndrome (SIRS) caused by trauma and injury.

~~When no high LPS concentration is present, then it was found that LBP does not affect TNF syntheses. However, the stimulation of macrophages by lower LPS quantities is inhibited by high LBP concentrations as they occur in vivo during the acute phase and can also be achieved by an exogenous addition of LBP.~~ **[Hereinafter the invention shall be explained in greater detail by an example and figures.]**

~~In accordance with this example of obtaining LBP, the~~ **[Obtaining LBP**

The] complete LBP cDNA is cloned in the pACHLT-B vector (Pharmingen, San Diego, USA) behind the strong polyhedrin promoter and behind glutathione S-transferase (GST) cDNA ~~to express\.~~ **[Thus]** a GST fusion protein **[is expressed]**. Then a 500 ~~ml~~ **[ml]** cell culture of Sf-9- insect cells is infected with this vector and the baculovirus DNA (Baculogold, Baculovirus DNA in a

linearized form, also from Pharmingen, San Diego, USA). After 2 days the cells are subjected to lysis and the lysate is coupled to glutathione sepharose in the presence of triton X-100 in a ~~batch~~ batch process. Then LBP is split off by the participant in the fusion by digestion with thrombin followed by a treatment with calbisorb to remove triton and a treatment with benzamidine sepharose to remove ~~the~~ thrombine ~~residue~~ [rests]. The resulting concentrations of pure LBP totals 0.3 – 0.5 ~~mg/ml~~ [mg/ml].

Legends to the figures

Figure 1: A murine macrophage cell line is stimulated in vitro with various concentrations of the bacterial toxin LPS for synthesizing the septicemia mediator TNF depending on LBP. In the case of high LPS concentrations not occurring in the organism LBP does not affect the synthesis of TNF. However, the stimulation of macrophages by lower LPS quantities is inhibited by high LBP concentrations as they occur in vivo during the acute phase and may be also achieved by an exogenous addition of LBP.

Figure 2: Here it appears that the addition of LBP in the presence of serum suppresses the production of TNF by the macrophage cell line. LBP contained in serum is responsible for this effect.

Figure 3: If the concentration of serum is increased with LBP added remaining constant the synthesis of TNF is also suppressed.

Figure 4: Here it is shown that LBP levels of the mouse produced by an exogenous addition of LBP correspond to the acute phase levels produced by addition of LPS, thus being physiological.

Figure 5: Cytokine release induced by LPS may be suppressed in the mouse by simultaneously adding LBP. A: TNF, B: IL-6.

Figure 6: In addition, the liver damage caused by LPS and detected by increasing ALT enzyme levels are suppressed by simultaneously adding LBP.

Figure 7: The addition of LBP reduces significantly the lethality in a LPS septicemia model, carried out with 20 mice per group.

Short interpretation:

Highly dosed LBP in vitro suppresses the synthesis of an important septicemia mediator molecule caused by the bacterial toxin LPS, namely TNF. The production of this protein and other mediators is suppressed in the mouse by simultaneously adding LBP. In addition, the liver damage induced by the addition of LPS is prevented by LBP and the number of surviving mice goes up significantly. That means, the addition of LBP seems to protect against the effects of LPS during septicemia, representing thus a new therapeutic principle for treating septicemia.